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JBIR-78 and JBIR-95: Phenylacetylated Peptides Isolated from *Kibdelosporangium* sp. AK-AA56

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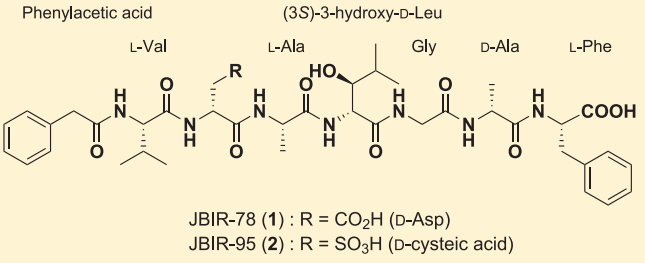
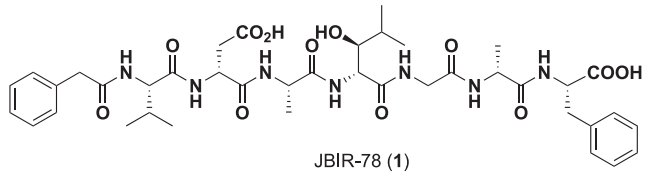
Supporting Information

ABSTRACT: The search for metabolites of *Kibdelosporangium* sp. AK-AA56 resulted in the discovery of novel *N*-phenylacetylated peptides, JBIR-78 (**1**) and JBIR-95 (**2**). Compounds **1** and **2** were established to be *N*-phenylacetylated heptapeptides by extensive NMR and HRESIMS analyses. The absolute configuration of the standard amino acids including a cysteic acid moiety was determined using Marfey's method on the acid hydrolysates of **1** and **2**. The relative and absolute configurations of a nonstandard amino acid, *β*-hydroxyleucine, were elucidated using the *J*-based and modified Mosher's methods, respectively. In an antimicrobial test, **1** showed antibacterial activity against *Micrococcus luteus*.

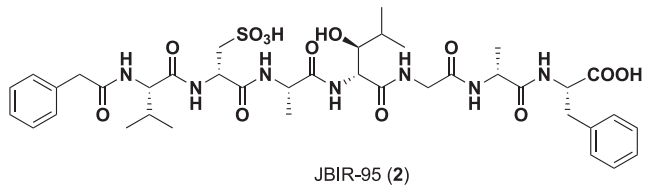


Members of the class Actinobacteria, particularly *Streptomyces*, have been extensively studied due to their ability to produce pharmaceutically useful compounds. However, the discovery of novel compounds from these bacteria has become more difficult. To increase our chance of discovering novel metabolites, we chose to examine rare actinomycetes. Accordingly, we isolated rare actinomycetes from a variety of sources such as soils and marine organisms and examined their secondary metabolites. During chemical screening, we discovered novel compounds including 20-membered macrolide ammocidins from *Saccharothrix* sp. AJ9571,[1] a heterobactin analogue, JBIR-16, from *Nocardia tenerifensis* NBRC 101015,[2] a diterpene, JBIR-65, from *Actinomadura* sp. SpB081030SC-15,[3] a BE-5221 analogue, JBIR-66, from *Saccharopolyspora* sp. SS81219JE-28,[4] and a macrocyclic dilactone, JBIR-101, from *Promicromonospora* sp. RL26.[5] Further screening revealed new *N*-phenylacetylated peptides, JBIR-78 (**1**) and JBIR-95 (**2**), from the culture of *Kibdelosporangium* sp. AK-AA56. This paper describes the production, isolation, and structural elucidation of **1** and **2**.

Kibdelosporangium sp. AK-AA56 was cultured in 100 mL of a production medium in 500 mL baffled Erlenmeyer flasks and incubated for 5 days at 27 °C on a rotary shaker. The mycelial cake was extracted with acetone and concentrated *in vacuo*. The aqueous concentrate was partitioned with EtOAc followed by extraction of the aqueous layer with *n*-BuOH. The resultant *n*-BuOH solution was concentrated *in vacuo* and subjected to sequential reversed-phase ODS flash column chromatography. Compounds **1** and **2** were finally purified using reversed-phase HPLC.



JBIR-78 (**1**)



JBIR-95 (**2**)

The structures of **1** and **2** were elucidated by spectroscopic methods including 2D NMR and tandem MS (MS/MS). The molecular formula of JBIR-78 (**1**) was determined to be $C_{40}H_{55}N_7O_{12}$. The peptidic structure of **1** was evident from the ^1H and ^{13}C NMR data recorded in DMSO-d_6, as shown in Table 1. Absorptions at 1650 and 1180 cm^{-1} in the IR spectrum of **1** revealed the presence of amide carbonyl groups. The ^1H and ^{13}C NMR spectra strongly suggested that **1** was a peptide compound. Detailed structural information was obtained from the HSQC, CT-HMBC,[6] and DQF-COSY spectra of **1** (Figure 1a).

The standard amino acid residues were identified as follows: a sequence from amide proton 2-NH (δ_H 8.19) to methylene protons H_2-23 (δ_H 3.08, 2.83) through an α-methine proton H-

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Table 1. ^{13}C (150 MHz) and ^{1}H (600 MHz) NMR Spectroscopic Data for JBIR-78 (1) and ^{13}C (125 MHz) and ^{1}H (500 MHz) NMR Spectroscopic Data for JBIR-95 (2) in DMSO-d_6

	JBIR-78 (1)		JBIR-95 (2)	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	173.2		173.1	
2	53.5	4.41, m	53.5	4.42, ddd (10.1, 8.3, 4.1)
2-NH		8.19, d (7.3)		8.14, d (8.3)
3	172.0		172.2	
4	48.0	4.27, dq (7.9, 7.0)	48.4	4.23, dq (7.3, 7.3)
4-NH		8.60, br s		7.69, d (7.3)
5	171.8		168.5	
6	42.6	3.71, dd (16.7, 5.9)	42.6	3.64, dd (15.4, 4.9)
		3.60, dd (16.7, 5.3)		3.56, dd (15.4, 6.1)
6-NH		7.81, dd (5.9, 5.3)		7.94, dd (6.1, 4.9)
7	168.4		171.7	
8	55.8	4.29, dd (8.2, 2.0)	56.7	4.30, dd (8.6, 2.2)
8-NH		7.89, d (8.2)		7.88, d (8.6)
9	172.9		172.8	
10	49.4	4.19, m	49.2	4.16, dq (7.1, 6.1)
10-NH		7.99, br s		7.93, d (6.1)
11	171.0		168.5	
12	50.0	4.55, ddd (5.9, 4.0, 2.3)	51.1	4.40, ddd (7.8, 6.0, 5.4)
12-NH		7.77, d (5.9)		8.58, d (6.0)
13	172.0		171.6	
14	59.7	3.90, dd (11.7, 7.2)	59.3	3.99, dd (12.2, 7.6)
14-NH		8.31, d (7.2)		8.32, d (7.6)
15	171.4		171.5	
16	41.9	3.54, d (14.1)	42.1	3.58, d (14.0)
		3.45, d (14.1)		3.47, d (14.0)
17	136.6		136.6	
18, 22	129.3	7.22, m	129.4	7.24, m
19, 21	128.4	7.23, m	128.4	7.25, m
20	126.5	7.17, m	126.6	7.18, m
23	37.3	3.08, dd (13.5, 3.8)	37.2	3.05, dd (13.5, 4.1)
		2.83, dd (13.5, 10.3)		2.85, dd (13.5, 10.1)
24	137.8		137.7	
25, 29	129.5	7.19, m	129.5	7.19, m
26, 28	128.4	7.22, m	128.4	7.22, m
27	126.6	7.25, m	126.7	7.17, m
30	18.8	0.97, d (7.0)	18.6	1.00, d (7.3)
31	76.3	3.53, dd (14.4, 2.0)	76.6	3.46, dd (13.9, 2.2)
32	30.6	1.56, dqq (14.4, 6.5, 6.5)	30.3	1.59, dqq (13.9, 6.5, 6.5)
33	19.5	0.89, d (6.5)	19.5	0.73, d (6.5)
34	19.1	0.72, d (6.5)	19.3	0.88, d (6.5)
35	17.6	1.05, d (7.0)	17.4	1.13, d (7.1)
36	36.4	2.70, dd (16.2, 4.0)	51.3	2.98, dd (12.8, 5.4)
		2.49, m		2.93, dd (12.8, 7.8)
37	172.1			
38	29.9	1.88, dqq (11.7, 6.8, 6.8)	29.8	2.04, dqq (12.2, 6.6, 6.6)
39	19.2	0.87, d (6.8)	19.4	0.81, d (6.6)
40	19.2	0.80, d (6.8)	18.3	0.84, d (6.6)

2 (δ_H 4.41, δ_C 53.5) was observed in the DQF-COSY spectrum. $^{1}H-^{13}C$ long-range correlations from the methylene protons H_2-23 to aromatic quaternary carbon C-24 (δ_C 137.8) and aromatic methine carbons C-25/29 (δ_C 129.5) were observed. Aromatic protons H-25/29 (δ_H 7.19) were strongly *meta*-coupled to aromatic methine carbon C-27 (δ_C 126.6). Although the signals for aromatic protons H-26/28 (δ_H 7.22) overlapped with those of another phenyl group, they were successfully assigned, as shown in Figure 1a, due to long-range coupling between H-26/28 and the aromatic quaternary carbon C-24. In addition, long-range coupling from the α-methine proton H-2 to amide carbonyl carbon C-1 (δ_C 173.2) proved the presence of a phenylalanine (Phe) residue.

The sequence from amide proton 4-NH (δ_H 8.60) to methyl protons H_3-30 (δ_H 0.97) through α-methine proton H-4 (δ_H 4.27, δ_C 48.0), which in turn was $^{1}H-^{13}C$ long-range coupled to a carbonyl carbon C-3 (δ_C 172.0), indicated the presence of an alanine residue (Ala1). Another alanine unit, Ala2, was similarly confirmed by $^{1}H-^{1}H$ correlations among amide proton 10-NH (δ_H 7.99), α-methine proton H-10 (δ_H 4.19, δ_C 49.4), and methyl protons H_3-35 (δ_H 1.05) as well as a $^{1}H-^{13}C$ long-range coupling between the α-methine proton H-10 and amide carbonyl carbon C-9 (δ_C 172.9).

A $^{1}H-^{1}H$ spin coupling between amide proton 6-NH (δ_H 7.81) and methylene protons H_2-6 (δ_H 3.71, 3.60) in combination with long-range coupling from H-6 to amide carbonyl carbon C-5 (δ_C 171.8) is indicative of a glycine substructure (Gly). A sequence from amide proton 12-NH (δ_H 7.77) to methylene protons H_2-36 (δ_H 2.70, 2.49), which in turn were long-range coupled to a carbonyl carbon C-37 (δ_C 172.1), through an α-methine proton H-12 (δ_H 4.55, δ_C 50.0) was observed in the DQF-COSY spectrum of **1**. The $^{1}H-^{13}C$ long-range couplings from the α-methine proton H-12 and the methylene protons H_2-36 to amide carbonyl carbon C-11 (δ_C 171.0) indicated that **1** contained an aspartic acid unit (Asp).

The last standard amino acid residue, a valine moiety (Val), was established as follows: two doublet methyl protons H_3-39 (δ_H 0.87) and H_3-40 (δ_H 0.80) spin-coupled to a methine proton H-38 (δ_H 1.88), indicating the presence of an isopropyl moiety. The $^{1}H-^{1}H$ spin correlation from amide proton 14-NH (δ_H 8.31) to the methine proton H-38 through α-methine proton H-14 (δ_H 3.90, δ_C 59.7) was observed in the DQF-COSY spectrum of **1**. In addition, the $^{1}H-^{13}C$ long-range couplings from the α-methine proton H-14 and the methine proton H-38 to amide carbonyl carbon C-13 (δ_C 172.0) confirmed the presence of a valine unit.

A nonstandard amino acid residue was also revealed by the interpretation of the DQF-COSY and CT-HMBC spectra. A sequence from amide proton 8-NH (δ_H 7.89) to methyl protons H_3-34 (δ_H 0.72) through α-methine proton H-8 (δ_H 4.29, δ_C 55.8), oxymethine proton H-31 (δ_H 3.53, δ_C 76.3), and methine proton H-32 (δ_H 1.56), which was also coupled to methyl protons H_3-33 (δ_H 0.89), was observed in the DQF-COSY spectrum. $^{1}H-^{13}C$ long-range couplings from the methine proton H-8 and the oxymethine proton H-31 to amide carbonyl carbon C-7 (δ_C 168.4) were observed in the CT-HMBC spectrum of **1**. As a result of these data, the presence of a nonstandard amino acid residue, β-hydroxyleucine (Hle), was established.

A remaining unit, a phenylacetic acid (Paa) moiety, was determined by $^{1}H-^{13}C$ long-range correlations from methylene protons H_2-16 (δ_H 3.54, 3.45) to the amide carbonyl carbon C-15 (δ_C 171.4), equivalent aromatic methine carbons C-18/22 (δ_C 129.3), and aromatic quaternary carbon C-17 (δ_C 136.6), which was coupled with aromatic protons H-19/21 (δ_H 7.23), and from the aromatic proton H-20 (δ_H 7.17) to aromatic carbons C-18/22.

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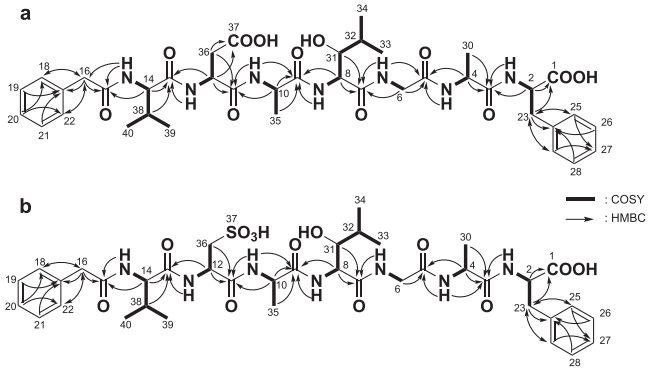

Figure 1. Key correlations observed in the 2D NMR spectra of **1** (a) and **2** (b) (bold lines show ^{1}H−^{1}H DQF-COSY results, and arrows show HMBC results).

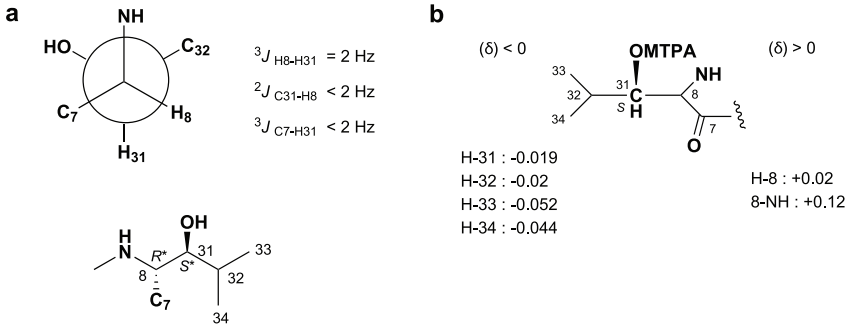

Figure 2. (a) Relative configuration of C-8 and C-31 in **1** determined using the *J*-based method. (b) Absolute configuration of C-31 in **1** determined using the modified Mosher's method. The differences in chemical shift values were obtained by subtracting the (*R*)-MTPA ester values from (*S*)-MTPA ester values (i.e., $\delta\Delta = \delta(S)$-MTPA − $\delta(R)$-MTPA).

To finalize the structure of **1**, the connectivity among these amino acid units and the Paa moiety was elucidated by the ^{1}H−^{13}C long-range couplings between 2-NH and C-3, 4-NH and C-5, 6-NH and C-7, 8-NH and C-9, 10-NH and C-11, 12-NH and C-13, and 14-NH and C-15. This amino acid sequence of **1** was further confirmed by HRESIMS/MS analysis (see Supporting Information Figure S7). Thus, **1** was identified as an *N*-phenylacetylated linear heptapeptide with a sequence of Paa−Val−Asp−Ala2−Hle−Gly−Ala1−Phe.

The molecular formula of JBIR-95 (**2**) was determined to be $C_{39}H_{55}N_7O_{13}S$, which suggested the presence of a sulfur-containing amino acid residue. Most of the NMR spectroscopic data for **2** were very similar to those of **1** (Table 1, Figure 1b). The sequence from amide proton 12-NH (δ_H 8.58) to methylene protons H_2-36 (δ_H 2.98, 2.93), which were also long-range coupled to an amide carbonyl carbon C-11 (δ_C 168.5), suggested the presence of a 2-amino-3-sulfopropanoic acid (Cya) unit. This was supported by HRESIMS/MS fragmentation analysis (see Supporting Information Figure S14). The collective spectroscopic data confirm that **2** comprises Paa−Val−Cya−Ala2−Hle−Gly−Ala1−Phe, as shown in Figure 1b.

The absolute configurations of the standard amino acid residues in **1** were determined using Marfey's method.[7] Since **1** contained two alanine residues, we performed partial acid hydrolysis to obtain separate fragments containing one Ala unit each prior to applying Marfey's method. The treatment of **1** with 6 N HCl at 40 °C for 6 h successfully gave two fragments, Hle−Gly−Ala1−Phe (**3**) and Paa−Val−Asp−Ala2−Hle−Gly (**4**). Fragments **3** and **4** were separately subjected to complete acid hydrolysis (6 N HCl, 120 °C for 14 h) followed by a reaction with N^{α}-(5-fluoro-2,4-dinitrophenyl)-L-alaninamide (FDAA) to obtain the respective FDAA derivatives. The retention times of these FDAA amino acid derivatives were established by HPLC monitoring with UV absorption at 340 nm and positive ion mode ESIMS. All derivatives were identified on the basis of a comparison of their retention times, molecular formulas, and UV spectra with those of standard amino acids derived from FDAA-conjugated compounds. The absolute configurations of the standard amino acid residues in **1** were identified as L-Phe, D-Ala1, L-Ala2, D-Asp, and L-Val. Prior to establishing the absolute configuration of the *β*-Hle unit, we used the *J*-based method[8] to determine the relative configuration. ^{1}H−^{13}C coupling constants were obtained from a *J*-resolved HMBC spectrum.[9] The small coupling constant ($^2J_{C31-H8}$ < 2 Hz) between C-31 and H-8 (δ_H 4.29) revealed that H-8 and the oxygen atom at C-31 are in the *anti* orientation, whereas the small coupling constant ($^3J_{H8-H31}$ = 2 Hz) between H-8 and H-31 suggested that these protons are *gauche*. Similarly, the small $^3J_{C7-H31}$ coupling constant (<2 Hz) indicated that C-7 and H-31 are also *gauche*, as shown in Figure 2a. Thus, the relative configurations at C-8 and C-31 were established as *R** and *S**, respectively (Figure 2a). The absolute configuration at C-31 in **1** was revealed using the modified Mosher's method.[10] The proton chemical shifts of the methine proton H-32 and the methyl protons H_3-33 and H_3-34 in the (*S*)-MTPA ester derivative of **1** were shifted upfield from those of the (*R*)-MTPA ester derivative (Figure 2b), while the proton chemical shifts of the *α*-methine proton H-8 and the amide proton 8-NH in the (*S*)-MTPA ester derivative were observed at a lower field than those of the (*R*)-MTPA ester derivative. Thus, the absolute configuration at the C-31

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position was deduced to be *S*. Combining these results, the absolute configuration of β-Hle was determined to be (*S*)-β-hydroxy-D-leucine. The absolute configurations of the amino acids in **2** were established in the same manner except for that of the Cya moiety. The absolute configuration of Cya in **2** was determined using N^{α}-(5-fluoro-2,4-dinitrophenyl)-L-valinamide (FDVA) instead of FDAA for Marfey's method, because FDAA derivatives of L-Cya and D-Cya were not separated by HPLC.[7] Accordingly, the Cya moiety was proven to be in the D-form. Therefore, the absolute structures of **1** and **2** were conclusively established.

Secondary metabolites produced by *Kibdelosporangium* were quite rare; approximately 30 compounds classified into only four groups including antibiotic YL 02107Q-A analogues,[11] aridicins,[12] kibdelones,[13] and cycloviracins,[14] have been reported. To the best of our knowledge, **1** and **2** were the first examples of peptides, which were aligned alternately with L and D forms of amino acids, from actinomycetes. In addition, although phenylacetyl *N*-terminal masked peptides from *Streptomyces* sp. such as antibiotic YF 044P-D,[15] antibiotic L 174580,[16] antibiotic YM 47690,[17] and JBIR-96[18] have been reported, *N*-phenylacetylated peptides, **1** and **2**, were isolated for the first time from *Kibdelosporangium*. The antimicrobial activity of **1** and **2** against *Micrococcus luteus*, *Candida albicans*, and *Escherichia coli* was examined. Only **1** was shown to be weakly active (inhibition zone, 7.1 mm) against *M. luteus*.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotation was measured on a Horiba SEPA-300 polarimeter. The UV spectra were measured on a Beckman Coulter DU730 UV/vis spectrophotometer. FT-IR spectra were obtained using a Horiba FT-720 spectrophotometer. ^{13}C (150 and 125 MHz) and ^{1}H (600 and 500 MHz) NMR spectra were recorded on a Varian NMR System 600 and 500 NB CL. The samples were measured in DMSO-d_6, and the solvent peak was used for spectra calibration (δ_C 39.7, δ_H 2.49 ppm). HRESIMS data were recorded on a Waters LCT-Premier XE mass spectrometer, and HRESIMS/MS data were recorded on a Waters SYNAPT G2. Reversed-phase medium-pressure liquid chromatography (MPLC) was performed on a Purif-Pack ODS 100 column (Shoko Scientific). Analytical and preparative reversed-phase HPLC were executed on an XBridge C_{18} column (5.0 μm, 4.6 i.d. × 150 mm; Waters) and XBridge C_{18} column (5.0 μm, 19 i.d. × 150 mm; Waters), respectively, with a Waters 2996 photodiode array detector and a Waters 3100 mass detector. The reagents and solvents were of the highest grade available.

Fermentation. *Kibdelosporangium* sp. AK-AA56 was isolated from a soil sample collected at Aomi, Tokyo Prefecture, Japan. The seed medium comprised 1% starch (Kosokagaku), 1% Polypepton (Nihon Pharmaceutical), 1% molasses (Dai-Nippon Meiji Sugar), and 1% meat extract (Extract Ehlrich, Wako Pure Chemical Industries) and was adjusted to pH 7.2 before sterilization. The production medium consisted of 2% glycerol, 1% molasses, 0.5% casein (Kanto Chemical), 0.1% Polypepton, and 0.4% $CaCO_3$ (Kozakai Pharmaceutical) and was adjusted to pH 7.2 before sterilization. Strain AK-AA56 was cultivated in 50 mL test tubes containing 15 mL of the seed medium. The test tubes were shaken on a reciprocal shaker (320 rpm) at 27 °C for 3 days. Aliquots (2.5 mL) of the culture were transferred to 500 mL Erlenmeyer flasks containing 100 mL of production medium and cultured on a rotary shaker (180 rpm) at 27 °C for 5 days.

Purification of 1 and 2. The fermentation broth (2 L) of AK-AA56 was centrifuged to obtain a mycelial cake, which was extracted with acetone (500 mL). The extract was concentrated *in vacuo*, and the residual aqueous concentrate was successively washed with EtOAc and extracted with *n*-BuOH. The *n*-BuOH layer was then concentrated *in vacuo*. The dried residue (660 mg) was subjected to reversed-phase MPLC using a MeOH−H_2O stepwise solvent system (0, 20, and 40% MeOH). The 20% MeOH eluate (38 mg) was further purified by preparative reversed-phase HPLC using an XBridge C_{18} column developed with 50% MeOH−H_2O containing 0.1% formic acid at a flow rate of 10 mL/min to isolate **1** (5.4 mg, t_R = 30.1 min) and **2** (14.2 mg, t_R = 12.4 min).

JBIR-78 (1): colorless amorphous solid; $[\alpha]^{25}_D$ +30.7 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 257 (3.76) nm; IR (KBr) ν_{max} 1650, 1560, 1180 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) and ^{13}C NMR (150 MHz, DMSO-d_6), see Table 1; HRESIMS *m/z* 826.4000 $[M + H]^+$ (calcd for $C_{40}H_{56}N_7O_{12}$, 826.3987).

JBIR-95 (2): colorless amorphous solid; $[\alpha]^{25}_D$ +12.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 257 (3.64) nm; IR (KBr) ν_{max} 1660, 1540, 1230, 1180, 1050 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6) see Table 1; HRESIMS *m/z* 862.3655 $[M + H]^+$ (calcd for $C_{39}H_{56}N_7O_{13}S$, 862.3657).

Determination of Amino Acid Configurations. Compound **1** (1.0 mg) was hydrolyzed in 0.2 mL of 6 N HCl at 40 °C for 6 h. After the reaction mixture was concentrated *in vacuo*, the dried residue was separated by reversed-phase HPLC using a CAPCELL PAK C_{18} MGII column (5.0 μm, 4.6 i.d. × 150 mm; Shiseido) developed with a gradient solvent system of 10−75% MeOH−H_2O containing 0.1% formic acid at a flow rate of 1 mL/min for 15 min to yield two fragments, **3** (t_R = 9.6 min) and **4** (t_R = 11.4 min). These fragments were characterized by HRESIMS data ($[M - H]^-$, *m/z* 434.2015, calcd for $C_{20}H_{28}N_5O_6$, 434.2040, for **3** and $[M - H]^-$, *m/z* 606.2784 calcd for $C_{28}H_{40}N_5O_{10}$, 606.2775, for **4**). Fragments **3** and **4** were hydrolyzed in 0.2 mL of 6 N HCl at 120 °C for 14 h. After the reaction mixtures were concentrated *in vacuo*, the residues were added to 0.1 M $NaHCO_3$ (200 μL) with 0.2 mg of FDAA or 0.2 mg of FDVA. The solutions were heated at 75 °C for 30 min. The FDAA derivatives of Phe, Asp, and Val were analyzed using an LC-MS system under the following conditions: column, CAPCELL PAK C_{18} MGII column; flow rate, 1 mL/min; solvent, 70% (for Phe), 40% (for Asp), or 60% (for Val) MeOH−H_2O containing 0.1% formic acid. The FDAA derivatives of Ala were analyzed with the LC-MS system using an ACQUITY UPLC BEH C_{18} column (2.1 i.d. × 100 mm; Waters) developed with 20% CH_3CN−H_2O containing 0.1% formic acid at a flow rate of 0.4 mL/min. The FDVA derivatives of Cya were also analyzed with the LC-MS system using a CROWNPAK CR(+) column (4.0 i.d. × 150 mm; DAICEL Chemical Industries) developed with 20% CH_3CN−H_2O containing 0.4% TFA at a flow rate of 1 mL/min. The retention times of the standard FDAA derivatives were as follows: L-Phe, 5.0 min; D-Phe, 7.9 min; L-Ala, 3.4 min; D-Ala, 5.6 min; L-Asp, 13.0 min; D-Asp, 18.7 min; L-Val, 6.2 min; and D-Val, 12.2 min. The retention times of the standard FDVA derivatives of Cya were 7.0 and 6.4 min for L-Cya and D-Cya, respectively. The retention times of the FDAA derivatives of **1** were as follows: Phe, 4.9 min; Ala1, 5.6 min; Ala2, 3.6 min; Asp, 18.9 min; and Val, 6.4 min. Compound **2** was hydrolyzed, derivatized with FDAA and FDVA, and analyzed in the same manner as **1**. The retention times of the FDAA and FDVA derivatives of **2** were as follows: Phe, 4.9 min; Ala, 5.5 and 3.7 min; Cya, 6.4 min; and Val, 6.4 min.

Compound **1** (1.0 mg) was reacted with (+)- or (−)-MTPA chloride (10 μL) in pyridine (200 μL) at room temperature for 14 h. The reaction mixture was concentrated to dryness, and the residue was dissolved in 10 mL of EtOAc−H_2O (1:1). The (*R*)- or (*S*)-MTPA ester recovered in the organic layer was dried *in vacuo* and purified by preparative reversed-phase HPLC using an XBridge C_{18} column (5.0 μm, 10 i.d. × 150 mm; Waters) with 75% MeOH−H_2O containing 0.1% formic acid at a flow rate of 4 mL/min to yield the (*R*)- or (*S*)-MTPA ester with t_R of 11.9 and 12.5 min, respectively.

Antimicrobial Activity. Antimicrobial activity against *M. luteus* was measured using the paper disk method. *M. luteus* was cultured in LB liquid medium consisting of 0.5% yeast extract (BD Biosciences), 1% tryptone (BD Biosciences), and 1% NaCl, at 28 °C for 24 h. A paper disk (diameter 6 mm, Whatman) that contained 10 μg of **1** or **2** was placed on an LB agar plate including 0.2% of the liquid culture; the plate was then incubated at 28 °C for 24 h. The antimicrobial activity is expressed as the diameter (mm) of the inhibitory zone. Erythromycin (10 μg) as a positive control showed an inhibition zone of 25 mm against *M. luteus*.

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■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H and ^{13}C NMR, DQF-COSY, HSQC, CT-HMBC, HRESIMS, and HRESIMS/MS spectra of **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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